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Paraoxonase 1 (PON1) deficiency in mice is associated with reduced expression of macrophage SR-BI and consequently the loss of HDL cytoprotection against apoptosis

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ABSTRACT

Background: Paraoxonase 1 (PON1) was shown to stimulate HDL binding and HDL-mediated cholesterol efflux from macrophages. This study examined the role of PON1 in the expression of proteins that enhance macrophage HDL binding, i.e. ABCA1 and SR-BI.

Methods and results: ABCA1 expression was similar, whereas SR-BI expression (mRNA and protein determined by FACS, Western blot, or immunocytochemistry) was significantly decreased in peritoneal macrophages from PON1 deficient (MPM-PON1⁰) in comparison to C57Bl/6 (MPM-Control) mice. PON1 deficiency correction with HDL-control, recombinant PON1 (rePON1), or by transfection with a plasmid containing the rePON1 gene, increased SR-BI expression in MPM-PON1⁰, whereas rePON1/H115Gln mutant, or the H115Q/H134Q double mutant, which lack catalytic activity, did not stimulate SR-BI expression. Lysophosphatidyl choline (LPC) resulting from PON1 action on macrophage PC, upregulated SR-BI expression in MPM-PON1⁰ via activation of ERK1/2 and PI3K. Functionally, HDL bound to MPM-PON1⁰ significantly less than to MPM-Control, and failed to inhibit tunicamycin-induced apoptosis, but had no significant effect on HDL-mediated cholesterol efflux from macrophages.

Conclusions: PON1 deficiency in mice is associated with decreased macrophage SR-BI expression, decreased cellular HDL binding, and consequently the loss of HDL-mediated cytoprotection against apoptosis, which may contribute to the accelerated atherosclerosis observed in PON1⁰ mice. These findings add new insights into the function of SR-BI in macrophages, and define the potential role of PON1 in regulating SR-BI-mediated HDL protection against macrophages apoptosis.

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1. Introduction

Serum paraoxonase 1 (PON1) is an HDL-associated lipolactonase and its activity is inversely related to the risk of cardiovascular diseases [1]. The role of PON1 in atherosclerosis was demonstrated in studies using mice lacking PON1 [2], or in mice over-expressing PON1 [3]. Anti-atherogenic actions of HDL-associated PON1 include protection of HDL [4] and of LDL [5] from oxidation, hydrolysis of specific oxidized lipids in lipoproteins [6], in atherosclerotic lesions [7], and in macrophages [8], stimulation of HDL binding to macrophages and HDL-mediated cholesterol efflux from macrophages [9].

Direct athero-cytoprotective effects of PON1 on macrophages were however less studied. PON1 was shown to decrease

macrophage cholesterol accumulation by inhibiting cholesterol biosynthesis [10] and attenuating oxidized LDL (Ox-LDL) uptake via the scavenger receptor CD-36 [11], and to directly reduce oxidative stress in macrophages [8]. Furthermore, we have shown that PON1 hydrolyzes macrophage phospholipids (phosphatidylcholine, PC) to yield lysophosphatidylcholine (LPC), which in turn stimulates HDL binding and cholesterol efflux from macrophages [12]. However, the detailed cellular mechanism responsible for these effects remains unexplored. Several cellular proteins, including ABCA1 and SR-BI, have been shown to contribute to HDL-mediated cholesterol efflux from macrophages [13]. In the present study we questioned whether PON1 have a direct impact on the expression of ABCA1 and SR-BI in macrophages and the possible functional implications.

2. Materials and methods

For detailed Materials and Methods please see the online-only supplement [Materials and Methods](#).

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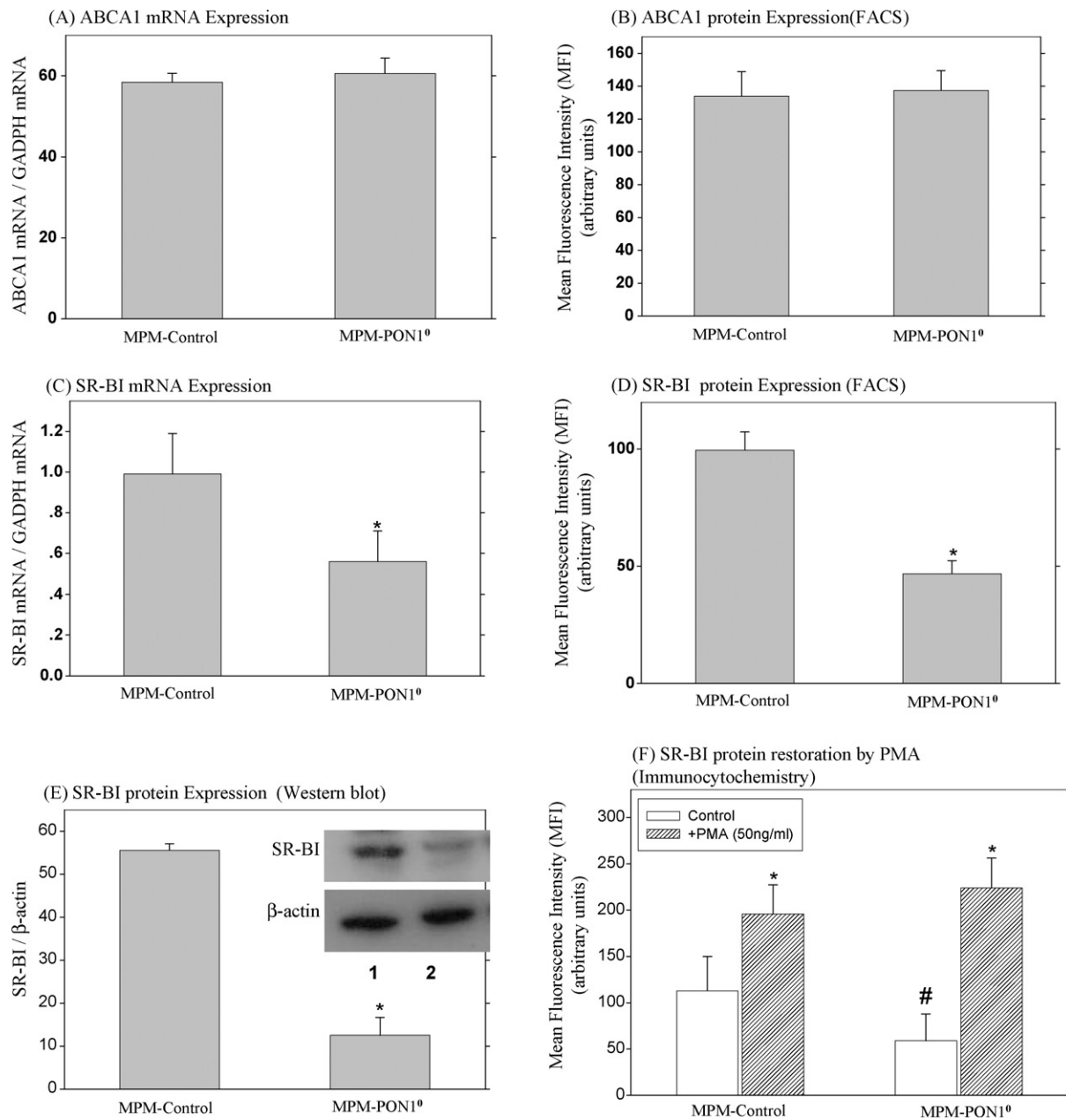


Fig. 1. ABCA1 and SR-BI expression in MPM from control and PON1⁰ mice. (A) ABCA1 mRNA; (B) ABCA1 protein; (C) SR-BI mRNA; (D) SR-BI protein analyzed by FACS, or by (E) Western blot. Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 MPM-PON1⁰ vs. MPM-Control. (F) SR-BI protein expression was analyzed in MPM grown on coated slides for 12 h in absence (Control) or presence of PMA (50 ng/ml), by immunocytochemistry using Confocal Bio-Rad system. Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 vs. control, #*p* < 0.01 MPM-PON1⁰ vs. MPM-Control.

2.1. Cells

J-774 A.1 murine macrophage-like cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD).

C57Bl/6 and PON1^{-/-} mice were sacrificed and peritoneal macrophages were harvested. The experimental protocol was approved by the Animal Care and Use Committee of the Technion, No. IL-046-04-2008.

2.2. Cell transfection

MPM were seeded at 0.8 × 10⁶ cells per 24 wells. Cells were transfected with the pET32b(+) plasmid containing the gene for rePON1-G2E6, using Nanofectin (PAA) as transfection reagent according to the manufactures' instructions.

2.3. Reverse transcriptase quantitative polymerase chain reaction (Q-PCR) for SR-BI and RT-PCR for ABCA1

Total RNA extraction and Q-PCR or RT-PCR reactions were carried out by routine protocols. Please see the online-only "Supplemental Materials and Methods" for details of primers and PCR protocols.

2.4. Macrophage SR-BI and ABCA1 protein expression

SR-BI protein was estimated by Western blot, by FACS and by immunocytochemistry, using specific anti-rabbit SR-BI antibodies. ABCA1 protein was estimated by FACS with anti-mouse FITC-conjugated ABCA1 antibodies. Please see online-only "Supplemental Materials and Methods" for details.

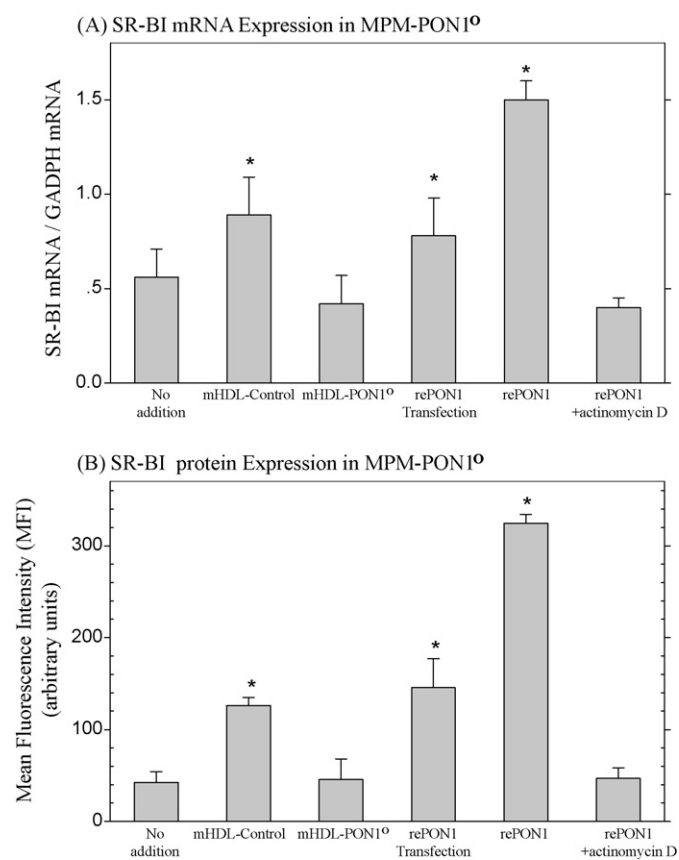


Fig. 2. PON1 increases macrophage SR-BI expression in MPM-PON1⁰. MPM from PON1⁰ mice were grown in the presence of HDL (100 μg protein/ml) from control mice (mHDL-Control), or from PON1⁰ mice (mHDL-PON1⁰), or were transfected with human rePON1, or incubated with recombinant PON1 (rePON1, 1.5 μg/ml) in absence or presence of actinomycin D (5 μg/ml). (A) SR-BI mRNA; (B) SR-BI protein. Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 vs. untreated cells (no addition).

2.5. HDLs preparation

Human HDL (hHDL) was isolated from healthy subjects, and mouse HDL (mHDL) was isolated from serum of control C57Bl/6 mice and from PON1⁰ mice, by continuous density gradient ultracentrifugation [14].

2.6. HDL binding to macrophages

FITC-conjugated hHDL or radiolabeled hHDL was incubated with macrophages at 37 °C for 1 h with. Lipoprotein cell association was then determined.

2.7. Determination of apoptosis

Apoptosis was measured by the loss of mitochondrial membrane potential detected by decreased emission from the dye 3,3'-dihexyloxycarbocyanine iodide (DiOC₆), and by annexin V-FITC and propidium iodide (PI) double staining (MEBCYTO Apoptosis Kit, MBL, Nagoya, Japan). Cell death was evaluated by measuring lactate dehydrogenase (LDH) in culture media using a commercially available kit.

2.8. Statistical analysis

Each separate experiment was performed in triplicate, and each individual experiment was replicated three times (*n* = 3). Sta-

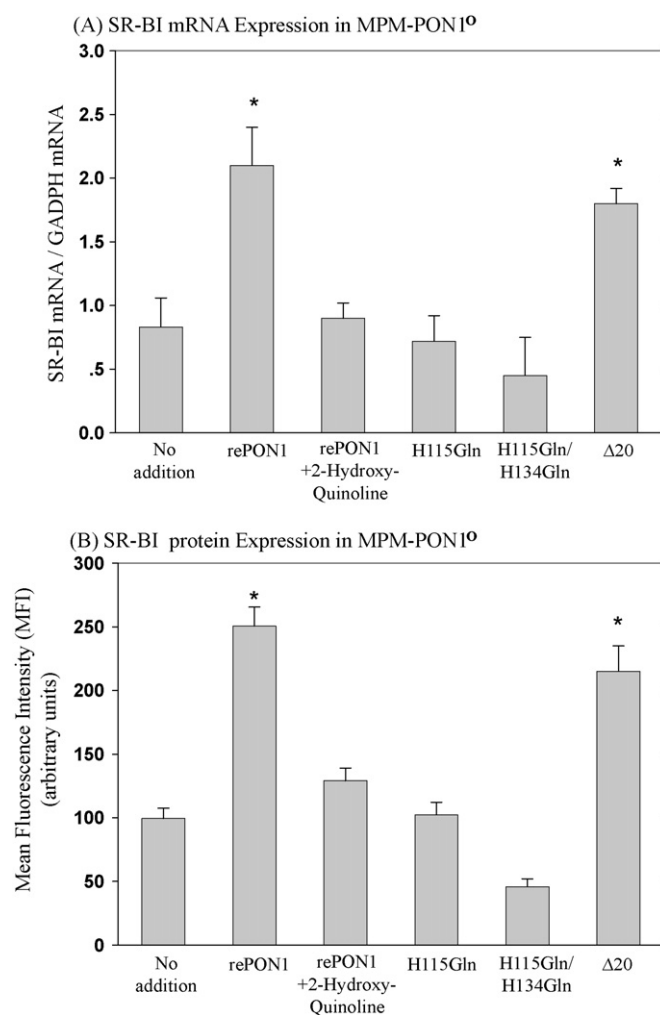


Fig. 3. PON1 catalytic activity is required for PON1-mediated increase in macrophage SR-BI expression. MPM-PON1⁰ were incubated for 18 h at 37 °C with DMEM medium supplemented with 0.2% BSA and rePON1 (1.5 μg/ml) in absence or presence of 2-hydroxyquinoline (200 μmol/L), or with rePON1 catalytic histidine dyad mutant H115Gln and with the double mutant H115Q/H134Q, or with rePON1 mutant lacking the 20 amino acids from the N-terminal region (PON1-Δ20) (1.5 μg protein/ml). (A) SR-BI protein; (B) SR-BI mRNA. Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 vs. untreated cells (no addition).

tistical analyses used Student's *t*-test for comparing differences between the two groups, and one-way ANOVA followed by the Student–Newman–Keuls test was used for comparing differences between multiple groups.

3. Results

3.1. ABCA1 and SR-BI expression in MPM from PON1⁰ mice

The expression of ABCA1 mRNA and protein was similar in MPM from both PON1⁰ and control mice (Fig. 1A and B). In contrast, SR-BI mRNA (Fig. 1C), as well as SR-BI protein, determined by either FACS (Fig. 1D) or Western blot (Fig. 1E) were significantly (*p* < 0.01) reduced by 49%, 50%, and by 76%, respectively, in MPM-PON1⁰, in comparison to MPM-Control. Immunocytochemistry analysis further confirmed that SR-BI protein expression is significantly (*p* < 0.01) lower, by twofold, in MPM-PON1⁰ in comparison to MPM-Control (Fig. 1F and Figure I of the online-only data supplement). Addition of PMA, which is a known inducer of SR-BI [15], increased the expression of SR-BI protein in MPM-Control by 72%. Surprisingly, however, PMA remarkably increased the expression of SR-BI

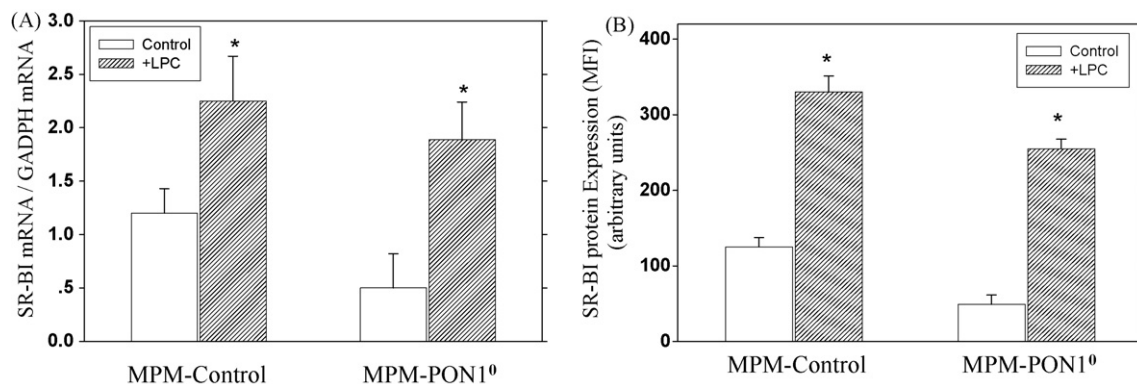


Fig. 4. LPC increases SR-BI expression in macrophages. MPM-Control and MPM-PON1⁰ were incubated for 18 h at 37 °C with DMEM medium supplemented with 0.2% BSA, in absence (Control) or presence of LPC (20 μmol/L). (A) SR-BI mRNA; (B) SR-BI protein. Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 LPC vs. Control.

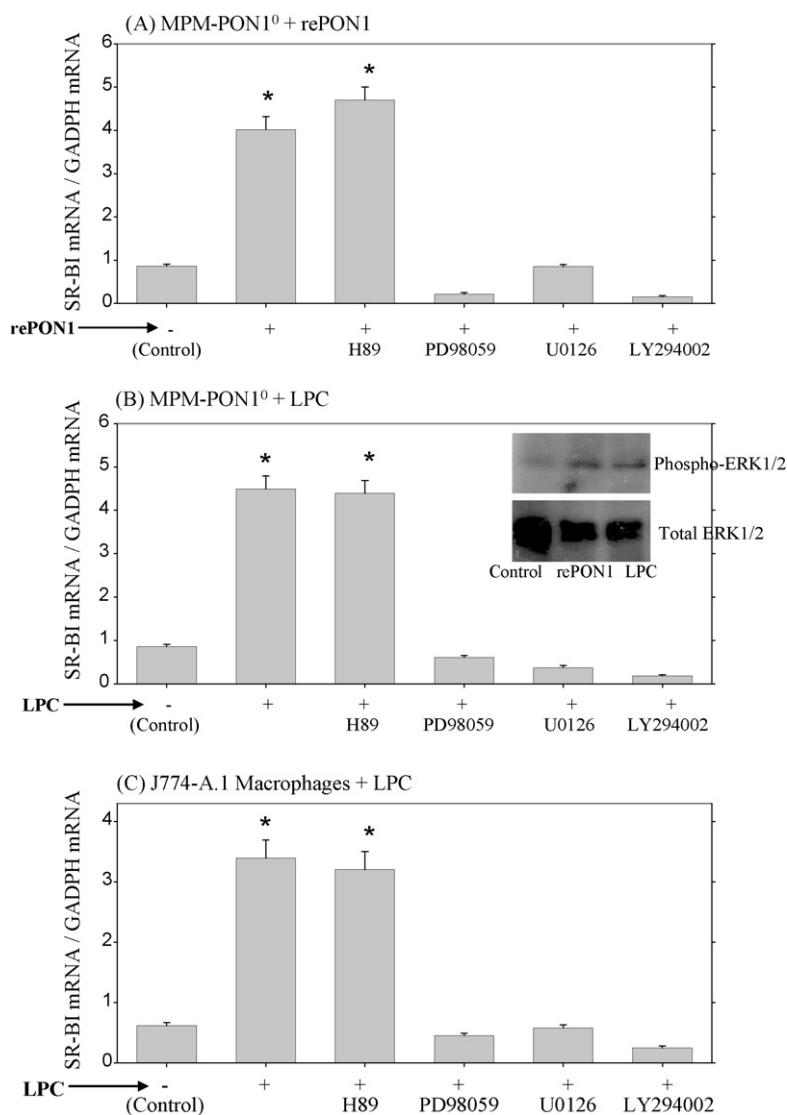


Fig. 5. PON1/LPC upregulate SR-BI mRNA expression via PI3K and ERK1/2 activation. SR-BI mRNA expression was measured in MPM-PON1⁰ incubated for 18 h at 37 °C with DMEM medium supplemented with 0.2% BSA, in absence (Control) or presence of rePON1 (1.5 μg/ml) (A), or LPC (20 μmol/L) (B), or in J774-A.1 Macrophages incubated with LPC (20 μmol/L) (C) in absence or presence of H89 (PKA inhibitor, 10 μmol/L), PD98059 (MEK1/2 inhibitor, 5 μmol/L), U0126 (MEK1/2 inhibitor, 10 μmol/L), or LY294002 (PI3K inhibitor, 5 μmol/L). Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 rePON1 or LPC treatment vs. Control. The inset in (B) shows a representative blot showing activation of ERK1/2 by rePON1 or LPC.

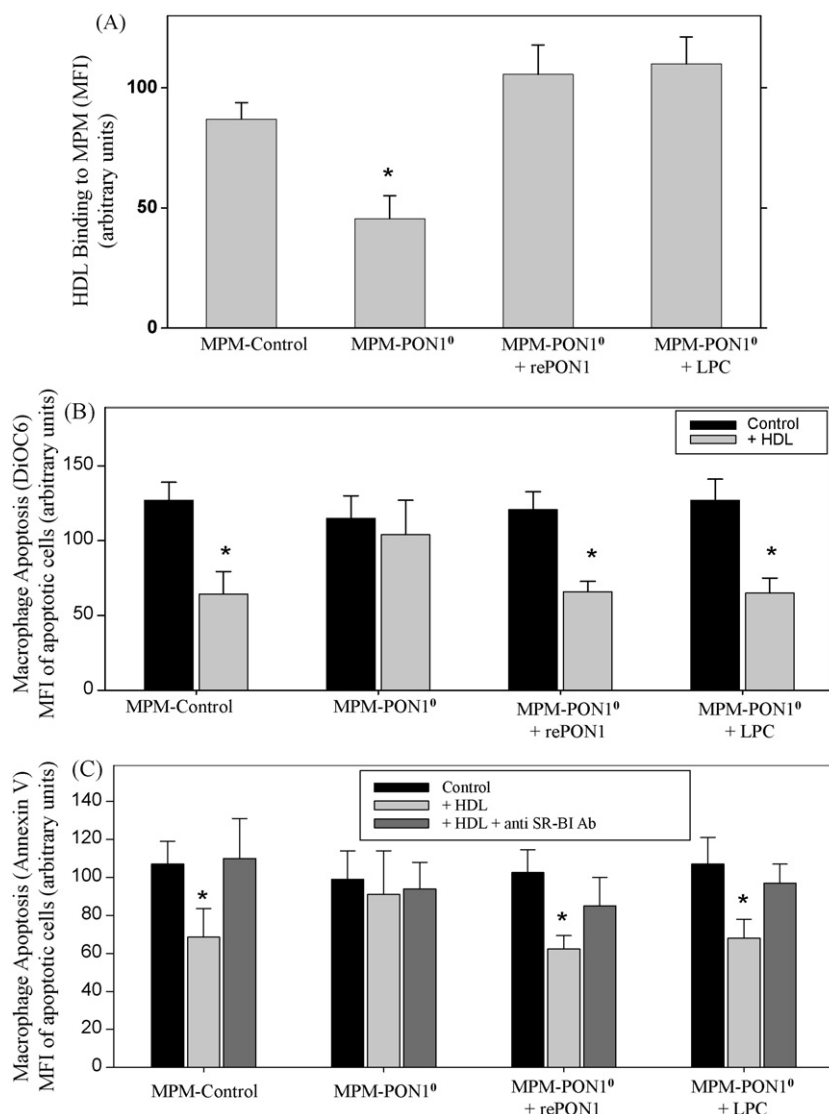


Fig. 6. PON1 mediates SR-BI-dependent HDL binding to macrophages and cytoprotective effect against apoptosis. HDL binding to MPM-Control and to MPM-PON1⁰ cultured in DMED medium supplemented with 0.2% BSA in absence or presence of rePON1 (1.5 μg protein/ml) or LPC (20 μmol/L) was determined by FACS after incubation of the cells for 1 h at 37 °C with FITC-conjugated HDL (10 μg protein/ml) (A). Results are expressed as the mean fluorescence intensity (MFI) ± SD of three separate experiments, 10,000 cells counted in each experiment. **p* < 0.01 vs. MPM-Control. Tunicamycin-induced macrophage apoptosis as detected by mitochondrial depolarization by DiOC₆ staining (B) or by annexin V staining (C) was measured in MPM-Control, MPM-PON1⁰, or MPM-PON1⁰ treated with rePON1 (1.5 μg protein/ml) or LPC (20 μmol/L), which were incubated in absence (Control) or presence of HDL (150 μg protein/ml) or (C) HDL together with anti-SR-BI blocking antibody. Results are expressed as mean ± SD of three separate experiments, **p* < 0.01 vs. Control.

protein also in MPM-PON1⁰, by up to 3.8-fold, to similar levels as expressed in PMA-treated MPM-Control (Fig. 1F), suggesting that the decreased expression of SR-BI in macrophages from PON1⁰ mice is restorable.

Analysis of SR-BI expression in the mice liver shows that hepatic expression of SR-BI protein is similar in control and in PON1-deficient mice, suggesting that the decreased expression of SR-BI in PON1-deficient mice is not a generalized effect.

3.2. PON1 restores SR-BI expression in MPM-PON1⁰

In order to examine whether correction of PON1 deficiency can restore SR-BI expression in macrophages, we exposed MPM-PON1⁰ to PON1 by alternative means. Incubation of MPM-PON1⁰ with mHDL derived from control mice (which contains PON1) increased the expression of SR-BI mRNA (Fig. 2A) and protein (Fig. 2B) in MPM-PON1⁰ by 59% and by threefold, respectively. On the contrary, mHDL from PON1⁰ (which lacks PON1) did not upregulate

macrophage SR-BI expression. Transfection of MPM-PON1⁰ with a pET32b(+) plasmid containing the rePON1-G2E6PON1 gene, also significantly (*p* < 0.01) increased SR-BI mRNA and protein expression by 40% and by 3.5-fold, respectively. Similarly, incubation of MPM-PON1⁰ with rePON1 significantly (*p* < 0.01) increased SR-BI mRNA and protein expression by 3- and 7.5-fold, respectively. However, incubation of the cells with rePON1 in the presence of actinomycin D, which interferes with gene transcription, abolished PON1-mediated increase in SR-BI expression (mRNA, as well as protein), suggesting that PON1 regulates SR-BI expression at the transcriptional level.

3.3. PON1 catalytic activity is required for PON1-mediated increase in macrophage SR-BI expression

To analyze whether PON1 catalytic activity is required for its effect on SR-BI expression in macrophages, MPM-PON1⁰ were incubated with rePON1 in the absence or presence of 2-

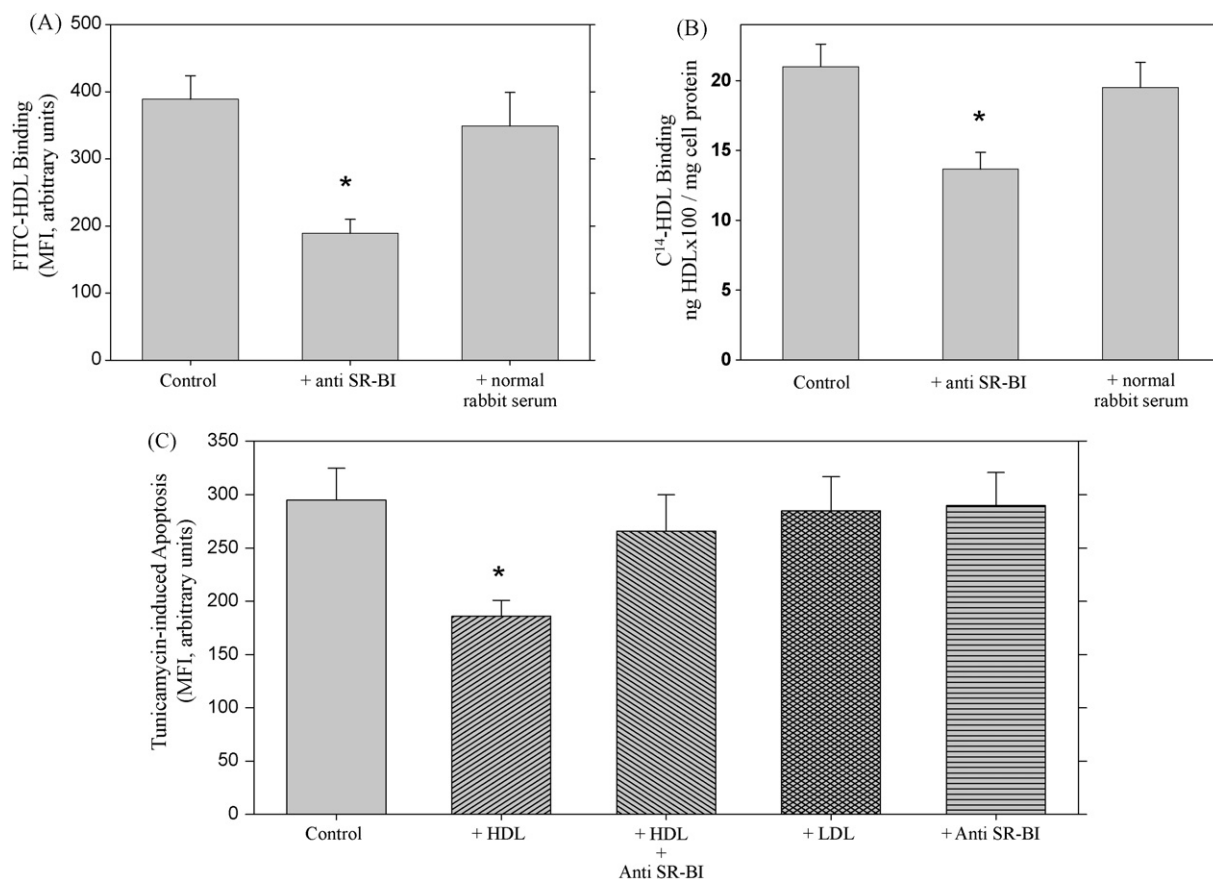


Fig. 7. SR-BI-dependent HDL binding to macrophages and cytoprotective effect against apoptosis. (A) J774-A.1 macrophages were incubated with FITC-conjugated hHDL in absence (Control) or presence of rabbit polyclonal anti-SR-BI (whole serum, blocking binding of ligands to SR-BI). Incubation with normal rabbit serum served as control. HDL binding to macrophages was measured by FACS. Results are expressed as the mean fluorescence intensity (MFI) \pm SD of three separate experiments, 10,000 cells counted in each experiment. * $p < 0.01$ vs. Control untreated cells. (B) J774-A.1 macrophages were incubated with cholesteryl C¹⁴-oleate labeled hHDL in absence (Control) or presence of rabbit polyclonal anti-SR-BI (whole serum, blocking binding of ligands to SR-BI). Incubation with normal rabbit serum served as control. HDL binding to macrophages was measured by radioactivity count as described in Section 2. (C) Tunicamycin-induced macrophage apoptosis in J774-A.1 that were incubated in absence (Control) or presence of HDL, HDL together with rabbit polyclonal anti-SR-BI (whole serum), and LDL, was detected by mitochondrial depolarization by DiOC₆ staining. Results are expressed as mean \pm SD of three separate experiments, * $p < 0.01$ vs. Control.

hydroxyquinoline, a specific inhibitor of PON1 activity. rePON1 increased SR-BI mRNA (Fig. 3A) and protein (Fig. 3B) expression by 2.5-fold, and addition of 2-hydroxyquinoline completely abolished this effect, suggesting that PON1 catalytic activity is required for PON1 effect on macrophage SR-BI expression. Furthermore, either rePON1 catalytic histidine dyad mutant H115Gln, or the double mutant H115Q/H134Q, which lack catalytic activity 9, did not induce any significant increase in macrophage SR-BI expression. On the contrary, cell incubation with rePON1 mutant lacking the 20 amino acids from the N-terminal region (rePON1- Δ 20), which lack HDL-binding ability [16], significantly ($p < 0.01$) increased SR-BI mRNA (Fig. 3A) and protein (Fig. 3B) expression by twofold, similarly to the effect of WT rePON1. Taken altogether, these results suggest that PON1 catalytic activity is required for stimulating SR-BI expression in macrophages.

We have previously shown that PON1 catalytic activity on macrophages can lead to the formation of LPC 10. To determine whether LPC mediates PON1-upregulation of macrophage SR-BI expression, MPM from control or from PON1⁰ mice were incubated with LPC. Fig. 4 shows that LPC significantly ($p < 0.01$) increased SR-BI mRNA (Fig. 4A) and protein (Fig. 4B) expression by 1.9- and 2.6-fold, respectively, in MPM-Control, and also in MPM-PON1⁰ (by 2.6- and 5.0-fold). Thus, LPC mimic the stimulatory effect of PON1 on macrophage SR-BI expression, suggesting that PON1-dependent LPC formation stimulates SR-BI expression in macrophages.

3.4. PON1/LPC stimulate macrophage SR-BI expression via ERK1/2 and PI3K signaling pathway

To elucidate signaling mechanisms involved in rePON1/LPC-mediated upregulation of SR-BI expression, we treated cells with rePON1 or LPC in the presence of PD98059 or U0126 that inhibit ERK1/2 activation, or LY294002, a PI3K inhibitor, or H89, a potent and selective inhibitor of PKA. Fig. 5A shows that PD98059, U0126, as well as LY294002, abrogated rePON1-stimulated expression of SR-BI in MPM-PON1⁰, whereas H89 had no effect, demonstrating thus the specificity of the ERK1/2 and PI3K as part of the LPC-induced signaling pathway. Similar results were obtained upon LPC treatment of MPM-PON1⁰ (Fig. 5B) or of J774-A.1 macrophages (Fig. 5C). In agreement, both rePON1 and LPC stimulated phosphorylation of ERK1/2 (Fig. 5B, inset). All inhibitors alone had no effect on SR-BI expression in macrophages (data not shown).

3.5. SR-BI is involved in the interaction of HDL with macrophages but does not affect cellular cholesterol efflux

To address the question whether HDL binds differently to MPM-PON1⁰ in comparison to MPM-Control, binding of FITC-conjugated hHDL was explored. hHDL binding to MPM-PON1⁰ was significantly ($p < 0.01$) reduced by 52% in comparison to hHDL binding to MPM-Control (Fig. 6A). However, pre-treatment of MPM-PON1⁰

with rePON1 or with LPC restored the capability of the cells to bind hHDL, to similar values obtained with MPM-Control. Because SR-BI has been implicated in cholesterol efflux, we examined next the hypothesis that the reduced HDL binding to MPM-PON1⁰ will reduce HDL-mediated cholesterol efflux from macrophages. Cholesterol efflux from MPM derived from PON1⁰ or control mice, was stimulated with hHDL (contains PON1) or with whole serum derived from PON1⁰ mice, which does not contain PON1), in order to eliminate any possible interaction of the cells with PON1. Surprisingly, no significant differences in cholesterol efflux were found among the two types of macrophages, when either hHDL (data not shown) or PON1⁰ mice-serum served as acceptors (Figure III of the online-only data) suggesting that SR-BI-mediated binding of HDL to macrophages does not contribute to cholesterol efflux from the cells under these experimental conditions.

3.6. PON1 mediates SR-BI-dependent HDL cytoprotective effect against apoptosis

HDL exerts its protective effect against atherosclerosis through several putative mechanisms, which extend beyond their involvement in cholesterol transport [17], including cytoprotection against apoptosis [18]. Thus, we tested next whether HDL influences apoptosis in MPM-PON1⁰ vs. MPM-Control. hHDL inhibited tunicamycin-induced apoptosis, measured by the loss of mitochondrial membrane potential (by up to 67%) in MPM-Control (Fig. 6B), but not in MPM-PON1⁰. Pre-treatment of MPM-PON1⁰ with either rePON1 or LPC, which were shown to restore SR-BI expression to normal levels (see Figs. 2 and 4), and cells' capability to bind hHDL (see Fig. 6A), also restored the capacity of hHDL to inhibit tunicamycin-induced apoptosis, suggesting that alterations in macrophage apoptosis associated with PON1 deficiency are due to reduced SR-BI expression. Moreover, staining by annexin V/PI clearly shows that indeed hHDL inhibit tunicamycin-induced apoptosis in MPM-Control but not in MPM-PON1⁰ (Fig. 6C). Control experiments with LDL (100 µg protein/mL), which was shown in previous studies to bind also to SR-BI and to mediate cholesterol efflux [19], demonstrated that this lipoprotein is not able to prevent apoptosis in either MPM-PON1⁰ or in MPM-Control.

Furthermore, incubation of the cells with either FITC-conjugated (Fig. 7A) or radiolabeled (Fig. 7B) hHDL in the presence of a rabbit polyclonal anti-SR-BI, which blocks binding to the receptor, abolished HDL-mediated protection against apoptosis in all experimental settings (Figs. 6C and 7C). These results provide direct evidence that alterations in macrophage apoptosis associated with PON1 deficiency are due to reduced SR-BI expression and consequently to reduced binding of HDL to these cells. Similar results were also obtained by using the macrophage cell line J-774A.1 (Fig. 7C). In all experiments described above the cells were PI negative, and no significant levels of LDH could be detected in cell media (data not shown), evidencing that the cells did not undergo necrosis.

4. Discussion

The present study shows for the first time that PON1 deficiency in mice is associated with reduced expression of SR-BI in macrophages. Functionally, this phenomenon resulted in lower capacity of the cells to bind HDL, and consequently lower HDL-mediated cytoprotection against apoptosis.

PON1 is an enzyme strongly associated with circulating HDL. PON1 deficiency was shown to be associated with increased macrophage cholesterol biosynthesis, cellular cholesterol accumulation, accelerated atherogenesis [10], vascular inflammation, thrombogenicity, expression of adhesion molecules, and enhanced

oxidative stress in the aorta [20] and in macrophages [8]. We have expanded these findings to show now that PON1 deficiency exposes the cells to reduced HDL-mediated cytoprotection against apoptosis. PON1 deficiency was associated with reduced SR-BI expression in macrophages, and consequently with reduced SR-BI-mediated HDL functions. We have shown previously that HDL-associated PON1 enhances HDL binding to macrophages [13]. Our present study shed light on the mechanism responsible for this effect, as we could demonstrate now that PON1-mediated increase in HDL binding to macrophages is related to variations in macrophage SR-BI expression. PON1, either when present as lipid-free PON1, or as HDL-associated PON1, stimulated SR-BI expression in macrophages at the transcriptional level. However, how PON1 deficiency can interfere with macrophage SR-BI expression is questionable. One possibility is through oxidative stress-related mechanism. PON1 deficiency is associated with increased macrophage oxidative stress [8], and SR-BI expression was previously found to be reduced by cell exposure to Ox-LDL [21].

In PON1⁰ mice SR-BI expression was significantly reduced in macrophages, and not affected the liver, suggesting that PON1 controls in a tissue-specific fashion the expression of SR-BI. Tissue-specific differences in SR-BI regulation were reported previously, in accordance with our present findings [22]. Different expression and regulation in different tissues may suggest different functional roles. In the liver SR-BI is primarily involved in reverse cholesterol transport and specific uptake of HDL-cholesterol, whereas in macrophages we demonstrate now a new role for SR-BI in HDL-mediated cytoprotection against apoptosis. The molecular basis for type-specific requirement of PON1 for SR-BI expression, however, is not understood yet.

Controversial data have been published regarding the implication of SR-BI in macrophage binding of HDL. We observed a remarkable reduction in HDL binding to macrophages from PON1⁰ mice that express lower levels of SR-BI in comparison to macrophages from control mice. Our results are in accordance with previous published studies by Lorenzi et al. [23], which directly evidenced that when the expression of SR-BI is reduced either by lipid loading with acetyl LDL, or by stimulation with 8-Br-cAMP, an analog of cyclic AMP, and also by specific siRNA transfection, the binding of HDL to macrophages decreases. In line with these results, Han et al. [24] showed that increased SR-BI expression induced by pitavastatin was associated with increased HDL binding to the macrophages. On the contrary, Brundert et al. [25] found no difference in HDL binding to macrophages with or without expression of SR-BI. It is possible that these differences stem from the different experimental setup of decreased expression vs. complete deficiency of SR-BI.

Although SR-BI is expressed in macrophages in both murine and human atherosclerotic lesions, its role in mediating cellular cholesterol efflux from macrophages is still controversial. The results of the present study show that despite a significant reduced expression of SR-BI in PON1⁰-MPM in comparison to Control-MPM, the cholesterol efflux from both types of cells was similar, suggesting that SR-BI-mediated binding of serum-HDL to macrophages does not contribute to cholesterol efflux from the cells. This is further supported by recent findings showing that SR-BI does not contribute to macrophage reverse cholesterol transport (RCT) *in vivo* [26]. Furthermore, treatment of PON1⁰-MPM with rePON1 or with LPC increased SR-BI expression, but did not increase cholesterol efflux. Apparently these results are inconsistent with our previous reports [13], however the experimental design of these studies differ in the stimulant of cholesterol efflux (HDL vs whole serum), in the cells used (the macrophage cell line J774-A.1 used in the previous study, which may respond differently than the primary MPM cells used in the present study), and in the LPC concentration, which was by twofold lower in the present study than that

employed with the J774-A.1 macrophages. Our results are sustained by previous studies showing that cholesterol efflux is similar in WT and in SR-BI knockout cells upon using HDL as an acceptor 26, suggesting an SR-BI-independent mechanism for cellular cholesterol removal. On the other hand, there is also a considerable amount of published evidence that SR-BI does facilitate the efflux of cellular cholesterol from macrophages to HDL [27]. We believe that these discrepancies stem from different experimental settings related to cell origin (human vs. murine) and to cellular cholesterol content. Nevertheless, our studies support a role for SR-BI in binding HDL to macrophages, and further investigations to evaluate SR-BI-mediated net flux of cholesterol in presence of HDL are necessary.

A large proportion of total cholesterol efflux from macrophages is mediated via the transporters ABCA1 and ABCG1 [28]. We did not find any significant change in macrophage ABCA1 expression in MPM from PON1⁰ mice vs. control mice, and these results may explain the similar HDL-mediated cholesterol efflux from MPM-PON1⁰ vs. MPM-Control.

Although the central anti-atherogenic activity of HDL is thought to be the removal of cholesterol from macrophage-foam cells, HDL has pleiotropic athero-protective properties, including the suppression of apoptosis [19]. Consistently, we show now that PON1 deficiency abrogates the cytoprotective effects of HDL, which are mediated via SR-BI.

The present study demonstrated that macrophage SR-BI expression is upregulated at the transcriptional level by PON1 via LPC formation. Furthermore, we could show that LPC increase SR-BI mRNA abundance via activation of PI3K and the mitogen-activated protein kinases (MAPK) ERK1/2. These results are in agreement with previous reports that implicated LPC in cellular transduction mechanisms of various genes [29]. However, more studies are needed to clarify detailed mechanisms and transcription factors involved in macrophage SR-BI gene activation by PON1-dependent LPC formation.

In conclusion, the findings of the present study may suggest a new role for PON1 in the cytoprotection of macrophages against apoptosis. PON1 may confer protection against macrophage apoptosis under basal conditions via LPC formation and further by upregulation of the macrophage SR-BI-mediated HDL binding to the cells. As macrophage apoptosis is an important feature of atherosclerotic plaque development, PON1 deficiency may lead to the enhanced atherosclerosis development observed in these mice, as a result of reduced SR-BI-mediated HDL protection against apoptosis.

Disclosures

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.01.025.

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